II. REMARKS

Preliminary Remarks:

In the specification:

In response to objections stated in the office action, the specification is amended as follows:

a) The title of the invention is changed to "Strand-Specific Polynucleotide Nickases," which is the type of nuclease that is described in the application.

b) The description of a photograph of an electrophoretic gel on page 18, line 7, is amended to refer to Figure 10, to which the description corresponds, rather than to Figure 3. The reference to Figure 3 was a typographical error.

In the claims:

Claims 16, 20, and 23 are amended, and new claims 30-32 are added.

Claim 16 is amended to correct an obvious typographical error.

Claim 20 is amended to be directed to a method that further comprises removing the nicked strand, *e.g.*, with an exonuclease (p. 9).

Claim 23 is amended to be directed to a method that further comprises producing a covalently closed linear molecule, *e.g.*, as described on pages 15-16.

New claim 30 is directed to a strand-specific polynucleotide nickase comprising a R.*Bpu*10I heteromeric restriction endonuclease which comprises first subunit and second subunits as defined in claim 1, wherein the second subunit is modified to render the catalytic domain thereof inactive, *e.g.*, as described in Example 1.

New claim 31 is directed to a process for producing a strand-specific polynucleotide nickase such as that to which claim 7 is directed, comprising inactivating the catalytic activity of one subunit of a heteromeric restriction endonuclease; *e.g.*, as described on page 6, lines 1-5.

New claim 32 is directed to a process for producing a strand-specific polynucleotide nickase, which process comprises inactivating the catalytic activity of one subunit of a heteromeric restriction endonuclease R.*Bpu*10I; also as described in Example 1.

The amendment and new claims are believed to contain no new matter.

Patentability Remarks:

Claims 16 and 23-25 were rejected under 35 U.S.C. §112, Second Paragraph, as being indefinite. In response, claims 16 and 23 are amended as follows:

a) The term "10of" is replaced with "of."

b) Claim 23 is amended to be directed to a method rather than to a "use."

The applicants respectfully disagree with the rejection of claims 24 and 25. The application clearly describes the claimed kit containing two polynucleotide nickase enzymes as defined in claim 1, one which nicks one strand of duplex DNA in a recognition sequence, and the other which nicks the other strand of duplex DNA in the same recognition sequence. For example, see the paragraph in the middle of page 10. The kit of claim 24 is directed to a kit for producing one or more site-specific nicks in pre-selected strands of a DNA duplex, comprising a first nickase as defined in claim 1, and a second nickase as defined in claim 1. The first and second nickase enzymes to which claims 24 and 25 refer are entities that are clearly distinct from the first and second nickase subunits to which claim 1 refers.

In view of the foregoing, withdrawal of the rejection of claims 16 and 23-25 under 35 U.S.C. §112, Second Paragraph, is respectfully requested.

35 U.S.C. §101

The rejection of claim 23 under 35 U.S.C. §101, for being directed to non-statutory subject matter (use according to claim 19) is obviated by the amendment of claim 23 to be directed to "[a] method according to claim 19."

35 U.S.C. §112, First Paragraph

Claims 1-8, 10-13, and 15-29 were rejected under 35 U.S.C. §112, First Paragraph, because the specification is enabling for endonuclease *Bpu*10I, but allegedly does not provide

enablement for the claimed invention wherein the nickase is an endonuclease other than *Bpu*10I.

The applicants respectfully traverse the rejection. The publication of Stankevicius *et al.* (1998) does not support the allegation that there may not be any other enzymes with the desired characteristics. In contrast, the first and second paragraphs of the introduction of this document indicate that there are a substantial number of type II restriction endonucleases that recognize asymmetric sequences, and of these there is a subgroup of enzymes (tentatively designated type ITT Enases) that cleave within the asymmetric recognition sequence. While *Bpu*10I was the first of this group to be fully characterized as having a heterosubunit structure, it is submitted that Stankevicius *et al.*, teaches that there are many other enzymes that are good candidates for having the same structure. Therefore, not only would the skilled person be aware that there was a strong likelihood for the existence of other enzymes with the desired characteristics, but they would also know exactly which enzymes had this potential. (A copy of Stankevicius *et al.* is attached for your ease of reference).

Turning to the disclosure of the present application, the skilled person is led towards the same enzymes by the teaching that the nickases of the present invention "recognise asymmetric nucleotide recognition sequences" (page 4, lines 5 to 6) and that "in a preferred embodiment the first subunit comprises a subunit from a type II restriction endonuclease" and "preferably a subunit from a heteromeric restriction endonuclease" (page 5, lines 12 to 14).

If the Examiner requires further evidence that a skilled person would reason in this manner, we would draw attention to US Patent Application Number US20030100094: Method for engineering strand-specific, sequence-specific, DNA-nicking enzymes, filed August 16, 2002. A copy of US20030100094 is attached. Please note that this patent application belongs to New England Biolabs, Inc., a competitor of Fermentas AB. We understand that it was filed in the United States on August 16, 2002 but claims priority from a provisional parent application filed on August 23, 2001.

The applicants of US20030100094 have apparently successfully practiced the present invention in creating nicking enzymes from *Bbv*CI restriction endonuclease. Specifically we would like to draw the Examiner's attention to paragraph [0030], which states "When the

hydrolysis sites are within the recognition sequence, or just outside, it seems unlikely that the enzyme would be symmetric, overall, but rather that it would be asymmetric, and would thus possess two different catalytic sites. We consider restriction endonucleases that recognize continuous, asymmetric sequences, and cleave within those sequences or very close to them, to be the ones most likely to possess two different catalytic sites. These enzymes are variously referred to as Type IIt or Type IIq endonucleases, as atypical Type IIs endonucleases." In the next paragraph the applicants of US20030100094 go on to indicate that 13 kinds of restriction endonucleases currently fall into this category; one of these is *Bbv*CI, another is *Bpu*10I.

Regarding the Examiner's second point, we would like to submit that US20030100094 provides good evidence that one of ordinary skill in the art could successfully practice the present invention with other enzymes, without the need for undue experimentation. Just as in the present application, the applicants for US20030100094 were able to identify a conserved motif within the sequence of a suitable enzyme, judge that the motif was likely to be the active site, create a mutation within the active site by site directed mutagenesis, test the activity of the mutant enzymes, and identify an enzyme with similar activity to that of R.*Bpu* 10I of the present invention (see US20030100094 - Example 1). The methods of experimentation and analysis practiced in US20030100094 are routine, and well known to the person skilled in the art. Accordingly, we would like to submit that the Examiner's allegation, that different enzymes may not be affected in the same way by mutation, is unfounded, and assert that the methods of the present invention are generally applicable. Accordingly, withdrawal of the rejection of claims 1-8, 10-13, and 15-29 under 35 U.S.C. §112, First Paragraph, is respectfully requested.

Conclusion

All rejections having been addressed, it is respectfully submitted that the present application is in condition for allowance and a Notice to that effect is earnestly solicited. If any points remain in issue, which the examiner feels may be best resolved through a personal

or telephone interview, he is kindly requested to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,

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Attachments:

1) Clean copy of Stankevecius et al. (1998) Nucleic Acids Research, 26(4):1084-1091.

2) Information Disclosure Statement, with Form PTO 1449 and copies of cited references.